

**AMENDMENTS TO THE CLAIMS**

The listing of claims will replace all prior listings of claims in the application.

Listing of claims:

**Claim 1 (previously presented):** A polysaccharide-protein conjugate or oligosaccharide-protein conjugate comprising an N-propionated polysaccharide or N-propionated oligosaccharide directly coupled to a protein through  $\beta$ -position sites of one or more propionate moieties of the N-propionated polysaccharide or N-propionated oligosaccharide; wherein the N-propionated polysaccharide or N-propionated oligosaccharide directly coupled to the protein elicits protective antibodies reactive with the N-propionated polysaccharide or N-propionated oligosaccharide; wherein the N-propionated polysaccharide or N-propionated oligosaccharide is de-N-acetylated and N-acryloylated; wherein at least 50% of the N-propionated polysaccharide or oligosaccharide is de-N-acetylated; and wherein the protein is a bacterial protein or a synthetic protein containing lysine or cysteine residues.

**Claim 2 (canceled).**

**Claim 3 (previously presented):** The conjugate according to claim 1 wherein the polysaccharide or oligosaccharide is obtained from bacteria, yeast, cancer cells, or is chemically synthesized.

**Claim 4 (previously presented):** The conjugate according to claim 1 wherein the polysaccharide or oligosaccharide is obtained from *Escherichia coli*, Meningococcus, Pneumococcus, Streptococcus, Neisseria, Salmonella, Klebsiella, or Pseudomonas.

**Claim 5 (previously presented):** The conjugate according to claim 1 wherein the polysaccharide or oligosaccharide is obtained from Group B *Streptococcus* selected from the

group consisting of type Ia, type Ib, type II, type III, type V, type VIII, and combinations thereof.

**Claim 6 (previously presented):** The conjugate according to claim 4 wherein the polysaccharide or oligosaccharide is derived from a *Meningococcus* group selected from the group consisting of group B, group C, group Y, group W135, and combinations thereof.

**Claim 7 (previously presented):** The conjugate according to claim 4 wherein the polysaccharide or oligosaccharide is derived from *E. coli* K1, *E. coli* K92, *Pneumococcus* type 4, *Pneumococcus* type 14, *Streptococcus* group A, *Streptococcus* group C, or combinations thereof.

**Claim 8 (previously presented):** The conjugate according to claim 1 wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, a *Neisseria meningitidis* outer membrane protein, pneumolysoid, C- $\beta$  protein from group B *Streptococcus* and non-IgA-binding C- $\beta$  protein from group B *Streptococcus*.

**Claim 9 (previously presented):** The conjugate according to claim 8 wherein the protein is recombinantly produced.

**Claim 10 (previously presented):** The conjugate according to claim 9 wherein the protein is recombinant *N. meningitidis* outer membrane protein.

**Claim 11 (previously presented):** The conjugate according to claim 1 wherein the polysaccharide or oligosaccharide comprises a glycosaminoglycan.

**Claim 12 (previously presented):** The conjugate according to claim 1 wherein the polysaccharide or oligosaccharide comprises glycosyl residues of a structural repeating unit having at least one free amino group or N-acyl group.

**Claim 13 (previously presented):** The conjugate according to claim 12 wherein the glycosyl residue is selected from the group consisting of glucosamine, galactosamine, mannosamine, fucosamine and sialic acid.

**Claim 14 (previously presented):** The conjugate according to claim 1 wherein the N-propionated polysaccharide or N-propionated oligosaccharide is directly coupled to an  $\epsilon$ -free amino group of a lysine residue or a thiol group of a cysteine residue of the protein.

**Claim 15 (previously presented):** The conjugate according to claim 1 wherein the polysaccharide or oligosaccharide is obtained from Group B *Streptococcus* type III, and wherein the protein is tetanus toxoid.

**Claim 16 (previously presented):** A polysaccharide-protein conjugate or oligosaccharide-protein conjugate comprising an N-propionated polysaccharide or N-propionated oligosaccharide directly coupled to a protein through  $\beta$ -position sites of one or more propionate moieties of the N-propionated polysaccharide or N-propionated oligosaccharide; wherein the conjugate elicits protective antibodies reactive with the N-propionated polysaccharide or N-propionated oligosaccharide, wherein said conjugate is produced by a method comprising:

A) de-N-acetylating an isolated polysaccharide or oligosaccharide using a de-N-acetylating reagent to form a de-N-acetylated polysaccharide or a de-N-acetylated oligosaccharide, wherein at least 50% of the N-propionated polysaccharide or N-propionated oligosaccharide is de-N-acetylated;

B) N-acryloylating the de-N-acetylated polysaccharide or the de-N-acetylated oligosaccharide with an acryloylating reagent to form an N-propionated polysaccharide or an N-propionated oligosaccharide, and

C) directly coupling through  $\beta$ -position sites of one or more propionate moieties of the N-propionated polysaccharide or the N-propionated oligosaccharide to a bacterial protein or a synthetic protein containing lysine or cysteine residues to form the polysaccharide-protein

conjugate or the oligosaccharide-protein conjugate.

**Claim 17 (previously presented):** The conjugate according to claim 16 wherein the polysaccharide or oligosaccharide is obtained from bacteria, yeast, cancer cells or is chemically synthesized.

**Claim 18 (previously presented):** The conjugate according to claim 16 wherein the coupling is conducted at a pH of about 7.0.

**Claim 19 (previously presented):** The conjugate according to claim 16 wherein the coupling is conducted at a pH above 9.

**Claim 20 (previously presented):** The conjugate according to claim 16 wherein the coupling is conducted in a reagent selected from the group consisting of phosphate buffer, bicarbonate buffer, and borate buffer.

**Claim 21 (previously presented):** The conjugate according to claim 16 wherein the de-N-acetylating reagent is a base or an enzyme and the acryloylating reagent is selected from the group consisting of N-acryloyl chloride, acryloyl anhydride, acrylic acid and a dehydrating agent.

**Claim 22 (previously presented):** A pharmaceutical composition comprising the conjugate according to any one of claim 1 and claim 16 and a pharmaceutically acceptable carrier.

**Claim 23 (original):** The pharmaceutical composition according to claim 22 further comprising an adjuvant.

**Claim 24 (original):** The pharmaceutical composition according to claim 23 wherein the adjuvant is selected from the group consisting of alum and stearyl tyrosine.

**Claim 25 (previously presented):** The pharmaceutical composition according to claim

22 further comprising a second immunogenic component, said second immunogenic component selected from the group of immunogens consisting of diphtheria-tetanus-pertussis (DTP), diphtheria-tetanus-acellular pertussis (DTaP), tetanus-diphtheria (Td), diphtheria-tetanus-acellular pertussis-*Haemophilus influenzae type B* (DTaP-Hib), diphtheria-tetanus-acellular pertussis-inactivated poliovirus-*Haemophilus influenzae type B* (DTaP-IPV-Hib), and combinations thereof.

**Claim 26 (previously presented):** An immunogen comprising the conjugates according to any one of claim 1 and claim 16, said immunogen elicits an N-propionated polysaccharide-specific or an N-propionated oligosaccharide-specific immune response.

**Claim 27 (previously presented):** The immunogen according to claim 26, wherein the immune response is generation of an N-propionated polysaccharide-specific or an N-propionated oligosaccharide-specific immunoglobulin.

**Claim 28 (original):** The immunogen according to claim 27 wherein the immunoglobulin is IgG, IgM, IgA or combinations thereof.

**Claim 29 (original):** A method of making a  $\beta$ -propionamido-linked polysaccharide-protein conjugate or a  $\beta$ -propionamido-linked oligosaccharide-protein conjugate comprising:

A) de-N-acetylating a polysaccharide or an oligosaccharide using a de-N-acetylating reagent to form a de-N-acetylated polysaccharide or de-N-acetylated oligosaccharide,

B) N-acryloylating the de-N-acetylated polysaccharide or de-N-acetylated oligosaccharide with an acryloylating reagent to form a  $\beta$ -propionated polysaccharide or a  $\beta$ -propionated oligosaccharide, and

C) directly conjugating the  $\beta$ -propionated polysaccharide or the  $\beta$ -propionamido oligosaccharide to a protein to form the  $\beta$ -propionamido-linked polysaccharide-protein or  $\beta$ -

propionamido-linked oligosaccharide-protein conjugate conjugate.

**Claim 30 (original):** The method of claim 29, wherein the de-N-acetylating reagent is a base or enzyme.

**Claim 31 (currently amended):** The method of claim 29 wherein the de-N-acetylating reagent is selected from the group consisting of NaOH, KOH and ~~KOH~~ LiOH.

**Claim 32 (original):** The method of claim 29, wherein the acryloylating reagent is selected from the group consisting of acryloyl chloride, acryloyl anhydride, acrylic acid and a dehydrating agent.

**Claim 33 (previously presented):** The method of claim 29, wherein the polysaccharide or oligosaccharide is obtained from bacteria, yeast, cancer cells or is chemically synthesized.

**Claim 34 (previously presented):** The method of claim 29 wherein the polysaccharide or oligosaccharide is obtained from *Escherichia coli*, Meningococcus, Pneumococcus, Streptococcus, Neisseria, Salmonella, Klebsiella, or Pseudomonas.

**Claim 35 (previously presented):** The method of claim 29 wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, a neisserial outer membrane protein, pneumolysoid, C- $\beta$  protein from group B Streptococcus and non-IgA binding C- $\beta$  protein from group B *Streptococcus*.

**Claim 36 (previously presented):** The method of claim 35, wherein the protein is recombinantly produced.

**Claim 37 (previously presented):** A vaccine comprising the conjugate according to any one of claim 1 and claim 16, wherein said vaccine provides protective immunity against at least one member of a genus of an organism from which the polysaccharide or oligosaccharide

component of the polysaccharide-protein conjugate or oligosaccharide-protein conjugate was obtained.

**Claim 38 (currently amended):** The vaccine according to claim 37 wherein the organism is selected from the group consisting of bacteria and<sub>1</sub> yeast, and cancer cell.

**Claim 39 (previously presented):** The vaccine according to claim 38 wherein the bacteria are selected from the group consisting of *Escherichia coli*, Meningococcus, Pneumococcus, Streptococcus, Neisseria, Salmonella, Klebsiella, and Pseudomonas.

**Claim 40 (previously presented):** The vaccine according to claim 37 further comprising a second immunogen in combination with the polysaccharide-protein conjugate or oligosaccharide-protein conjugate, said second immunogen selected from the group consisting of diphtheria-tetanus-pertussis (DTP), diphtheria-tetanus-acellular pertussis (DTaP), tetanus-diphtheria (Td), diphtheria-tetanus-acellular pertussis-*Haemophilus influenzae type B* (DTaP-Hib), diphtheria-tetanus-acellular pertussis-inactivated poliovirus-*Haemophilus influenzae type B* (DTaP-IPV-Hib), and combinations thereof.

**Claim 41 (original):** A method of immunizing a mammal against a disease causing organism or disease causing cell comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.

**Claim 42 (original):** A method of immunizing a mammal against *Streptococcus pneumoniae* comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.

**Claim 43 (original):** A method of immunizing a mammal against Group B *Streptococcus* comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.

**Claim 44 (original):** A method of immunizing a mammal against Group B *Neisseria meningitidis* comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.

**Claim 45 (original):** A method of immunizing a mammal against Group C *Neisseria meningitidis* comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.

**Claim 46 (original):** A method of immunizing a mammal against *Haemophilus influenzae* type B comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.

**Claim 47 (previously presented):** A method of eliciting an antibody response to a polysaccharide or an oligosaccharide in a mammal comprising administering an effective amount of the conjugate according to any one of claim 1 and 16.

**Claim 48 (original):** An immunoglobulin or antigen-binding fragment thereof produced according to the method of claim 47.

**Claim 49 (original):** The immunoglobulin according to claim 48, selected from the group consisting of IgG antibody, IgM antibody, IgA antibody and combinations thereof.

**Claim 50 (original):** The immunoglobulin according to claim 49, wherein the antibody is an isolated IgG.

**Claim 51 (previously presented):** An isolated antibody or antigen binding fragment thereof elicited in response to the  $\beta$ -propionamido-linked polysaccharide-protein conjugate or  $\beta$ -propionamido-linked oligosaccharide-protein conjugate according to any one of claim 1 and 16, said antibody or antigen fragment thereof specifically immunoreactive with N-propionated polysaccharide or N-propionated oligosaccharide and immunoreactive with a native N-acetylated



polysaccharide from which the  $\beta$ -propionated polysaccharide or  $\beta$ -propionated oligosaccharide was obtained.

**Claim 52 (previously presented):** The antibody or antigen binding fragment thereof according to claim 51 wherein the native N-acetylated polysaccharide is obtained from bacteria, yeast, cancer cells, or is chemically synthesized.

**Claim 53 (previously presented):** The antibody or antigen binding fragment thereof according a claim 52 wherein the polysaccharide is obtained from *Escherichia coli*, Meningococcus, Pneumococcus, Streptococcus, Neisseria, Salmonella, Klebsiella, or Pseudomonas.

**Claim 54 (original):** The antibody or antigen binding fragment thereof according to claim 51 wherein the antibody is recombinantly produced.

**Claim 55 (previously presented):** A method of passive immunization against a disease causing organism or disease causing cells comprising administration of an effective amount of the immunoglobulin or antibody according to claim 48, said amount is sufficient to inhibit or kill the disease causing organism or disease causing cells.

**Claim 56 (original):** The method of passive immunization according to claim 55 wherein the immunoglobulin is an isolated IgG antibody or antigen binding fragment thereof.

**Claim 57 (original):** The method of passive immunization according to claim 55 wherein the immunoglobulin is an isolated IgM antibody or antigen binding fragment thereof.

**Claim 58 (previously presented):** The method of passive immunization according to claim 55 wherein the immunoglobulin is an isolated IgA antibody or antigen binding fragment thereof.

**Claim 59 (currently amended):** The conjugate according to claim 1, wherein the de-

N-acetylated polysaccharide or the de-N-acetylated oligosaccharide is at least 95% N-acryloylated.

**Claim 60 (previously presented):** The conjugate according to claim 16, wherein the de-N-acetylated polysaccharide or the de-N-acetylated oligosaccharide is at least 95% N-acryloylated.

**Claim 61 (previously presented):** The conjugate according to any one of claim 1 and claim 16, wherein the bacterial protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, cholera toxin subunit B, *Neisseria meningitidis* outer membrane proteins, pneumolysoid, C- $\beta$  protein from group B *Streptococcus*, *Pseudomonas aeruginosa* toxoid, and pertussis toxoid.

**Claim 62 (previously presented):** A method of passive immunization against a disease causing organism or disease causing cells comprising administration of an effective amount of the immunoglobulin or antibody according to claim 51, said amount is sufficient to inhibit or kill the disease causing organism or disease causing cells.

**Claim 63 (previously presented):** The method of passive immunization according to claim 62 wherein the immunoglobulin is an isolated IgG antibody or antigen binding fragment thereof.

**Claim 64 (previously presented):** The method of passive immunization according to claim 62 wherein the immunoglobulin is an isolated IgM antibody or antigen binding fragment thereof.

**Claim 65 (previously presented):** The method of passive immunization according to claim 62 wherein the immunoglobulin is an isolated IgA antibody or antigen binding fragment thereof.